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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: EVANS, et al.

Title: NOVEL STEROID-ACTIVATED NUCLEAR RECEPTORS AND USES THEREFOR

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CERTIFICATE OF MAILING

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AMENDMENT

Commissioner for Patents
Box Missing Parts
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Sir:

In response to the Office communication dated March 19, 2002 (the Notice to File Corrected Application Papers), please consider the following amendments and remarks.

In the Specification

Please replace paragraph [0024] with the following amended paragraph [0024]. A marked-up version of this paragraph, showing the changes made, is also provided herewith as Appendix A.

-- [0024] Figures 1A & 1B show the sequence of the longest SXR cDNA clone (SEQ ID NO: 1) and a corresponding encoded protein (amino acids 41-434 of SEQ ID NO: 2). The DNA binding domain (amino acids 41-107) is shown in bold, and upstream termination codons in frame with the putative initiator leucine are indicated by asterisks. That this Leu can function as an initiator was demonstrated by SDS-PAGE analysis of labeled proteins produced from *in vitro* transcribed, translated cDNAs. The unmodified cDNAs yielded a translation product

indistinguishable in size from that produced when the leucine was changed to methionine, albeit not nearly as efficient. - -

Please replace paragraph [0025] with the following amended paragraph [0025]. A marked-up version of this paragraph, showing the changes made, is also provided herewith as Appendix A.

-- [0025] Figure 1C presents a schematic comparison between SXR and other RXR partners (e.g., the *Xenopus* benzoate X receptor (xBXR), the human vitamin D3 receptor (hVDR), the human constitutively active receptor-alpha (hCARα), the rat farnesoid X receptor (rFXR), the human peroxisome proliferator activated receptor alpha (hPPARα), the human liver-derived receptor X (LXRα), the human retinoic acid receptor alpha-1 (hRARα-1), the human thyroid hormone receptor beta (hTRβ), the human retinoid X receptor alpha (RXRα) and the human glucocorticoid receptor alpha (hGRα)). Ligand-binding domain boundaries follow those for the canonical nuclear receptor ligand-binding domain (Wurtz et al., *Nature Struct. Biol.* 3:87-94, 1996). Similarity between RXR and other receptors is expressed as percent amino acid identity (indicated in Arabic numerals above each clone). Amino acid residues in the sequences were aligned using the program GAP (Devereaux et al., *Nucl. Acids Res.* 12:387-395, 1984). DNA = DNA binding domain and LIGAND = ligand binding domain. - -

Please replace paragraphs [0030] and [0031] with the following amended paragraphs [0030] and [0031]. A marked-up version of these paragraphs, showing the changes made, is also provided herewith as Appendix A.

-- [0030] Figure 6 collectively is a series of illustrations indicating that SXR can activate responsive elements found in various steroid and xenobiotic inducible P450 enzymes.

[0031] Figure 6A presents a schematic comparison of nucleotide sequences encoding response elements found in inducible cytochrome P450 enzymes. A database search for repeats

of the sequence RGKTCA (SEQ ID NO: 41) was performed and some of the matches for enzymes involved in hepatic steroid hydroxylation are indicated. The standard nomenclature for P450 enzymes has been utilized. P450R is the single P450 oxidoreductase required for hydroxylation of steroids. UGT1A6 is a rat uridine diphosphate (UDP)-glucuronosyltransferase that conjugates glucuronic acid to hydroxylated steroids. - -

Please replace paragraph [0039] with the following amended paragraph [0039]. A marked-up version of this paragraph, showing the changes made, is also provided herewith as Appendix A.

-- [0039] Figure 8C illustrates that the DR-3 element is essential for SXR-mediated activation of CYP3A23, and is interchangeable with the IR-6 element. The wild type (DR3/WT (SEQ ID NO: 39), filled bars) or mutant forms (DR3/M1 (SEQ ID NO: 42), open bars; DR3/M2 (SEQ ID NO: 43), stippled bars; and DR3/IR6, hatched bars) of CYP3A23 cellular promoter reporters were transfected into primary rat hepatocytes in the presence of expression vector for SXR. The ligand treatment and data presentation are the same as in 8A. RIF, rifampicin; CTZ, clotrimazole. Note the disruptions of DR-3 element (DR3/M1, and DR3/M2) abrogate the activation of CYP3A23, and the replacement of DR-3 element with IR-6 element (DR3/IR3) rescue the responsiveness. - -

Please replace paragraph [0145] with the following amended paragraph [0145]. A marked-up version of this paragraph, showing the changes made, is also provided herewith as Appendix A.

-- [0145] Mutations in the IR-M nucleotide sequence prevented binding of the heterodimer to the response element.

CYP3A oligonucleotides tested had the following sequences:

CYP3A4,tagaataTGAACTcaaaggAGGTCAgtgagtgg (SEQ ID NO:33);

CYP3A5, tagaataTGAACTcaaaggAGGTAAgcaaaggg (SEQ ID NO:34); and

CYP3A7, tagaataTTAACTcaatggAGGCAgtgagtgg (SEQ ID NO:35). - -

Please replace paragraph [0151] with the following amended paragraph [0151]. A marked-up version of this paragraph, showing the changes made, is also provided herewith as Appendix A.

-- [0151] The fact that SXR is necessary and sufficient to render the induction of both human CYP3A4 and rat CYP3A23 gene in rodent hepatocytes by RIF suggested that the host cellular environment, SXR/PXR herein, rather than the gene structure, dictates the patterns of inducibility of CYP3A genes. The above notion would predict: (1) The SXR/PXR response element is essential for the activation of CYP3A genes; and (2) The response elements of SXR and PXR are interchangeable. Therefore, mutagenesis analysis was performed on the promoter of the rat CYP3A23 gene to examine these predictions. In vitro electrophoretic mobility shift assays showed that both SXR:RXR and PXR:RXR heterodimers efficiently bind to the DR-3 element (5' TGAACTtcaTGAACT 3' (SEQ ID NO: 39)) in the CYP3A23 promoter (Blumberg et al., 1998, 1998). As shown in Figure 8C, mutation of both half sites (DR3/M1) or a single half site (DR3/M2) abolished the PXR and/or SXR-mediated activation by PCN, RIF, and CTZ; On the other hand, replacement of the wild type DR-3 element by an IR-6 element of the human CYP3A4 gene promoter (Blumberg et al., 1998, and Kliewer et al., 1998) successfully rescue the inducibility by PCN, RIF and CTZ. - -

Please replace paragraph [0154] with the following amended paragraph [0154]. A marked-up version of this paragraph, showing the changes made, is also provided herewith as Appendix A.

-- [0154] Genomic DNA was isolated as described before (Xie et al. 1999). The polymerase chain reaction (PCR) was used to screen the transgene positive mice. Two

oligonucleotides used to screen Alb-SXR mice are 5'-GAGCAATTCGCCATTACTCTGAAGT-3' (SEQ ID NO: 36, annealing to SXR cDNA), and 5'-GTCCTTGGGGTCTTCTACCTTTCTC-3' (SEQ ID NO: 37, annealing to the SV40 sequence downstream of the transgene in the transgene cassette). Another two oligonucleotides used to screen Alb-VPSXR are 5'-GACGATTTGGATCTGGACATGTTGG-3' (SEQ ID NO: 38, annealing to VP16 sequences), and 5'-GTTTTCATCTGAGCGTCCATCAGCT-3' (SEQ ID NO: 40, annealing to the SXR cDNA). PCR was carried out in a DNA thermal cycler (Perkin-Elmer/Cetus) using the following program: 94 °C for 1 min, 58 °C for 2 min, and 72 °C for 3 min and products were analyzed by electrophoresis on a 1% agarose gel. The transgene integration status was analyzed by Southern blot using transgene specific probes as described before (Xie et al. 1999). - -.

Sequence Listing

Applicants respectfully submit a computer readable form (CRF) and a paper copy of the sequence listing herewith. A statement to support filing and submission in accordance with 37 C.F.R. 1.821-1.825 is also enclosed herewith.

In the Drawings

Please replace all of the originally filed drawings with the enclosed fifteen (15) sheets of substitute drawings.